

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460



OFFICE OF CHEMICAL SAFETY AND  
POLLUTION PREVENTION

**MEMORANDUM**

**Date:** February 13, 2013

**SUBJECT:** Ethylenethiourea, Immunotoxicity study in Rats

**PC Code:** 600016

**Decision No.:** NA

**Petition No.:** N/A

**Risk Assessment Type:** N/A

**TXR No.:** 0056580

**MRID No.:** 48794502, 48794501, 48807701

**DP Barcode:** 402788

**Registration No.:** N/A

**Regulatory Action:** N/A

**Submission No.:** N/A

**CAS No.:** 96-45-7

**40 CFR:** N/A

**FROM:** Yung G. Yang, Ph.D.  
Risk Assessment Branch VI  
Health Effects Division (7509 P)

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**THROUGH:** Felecia Fort, Chief  
Risk Assessment Branch VI  
Health Effects Division (7509 P)

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**TO:** Christina Scheltema  
RMIB3  
Pesticide Re-Evaluation Division (7508P)  
And  
Michael Metzger, Chief  
Risk Assessment Branch VII  
Health Effects Division (7509P)

**I. CONCLUSIONS**

The immunotoxicity study in rats for Ethylenethiourea (MRID 48794502) has been reviewed. It is classified as acceptable/guideline and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800).

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## II. BACKGROUND and ACTION REQUESTED

An immunotoxicity study on Ethylenethiourea (MRID 48794502) has been submitted. RAB VI was asked to review and prepare a DER for this study.

## III. RESULTS AND DISCUSSION

The immunotoxicity study in mice for Ethylenethiourea (MRID 48794502) has been reviewed. The DER is attached and an executive summary is as follows:

**EXECUTIVE SUMMARY:** In an immunotoxicity study (MRID #48794502), Ethylenethiourea (100 % w/w a.i., Batch no. 07202TD) was administered to Male Wistar rats (10/dose) in the diet at dose levels of 0, 10, 50, or 250 ppm (equivalent to 0, 1, 4, and 19 mg/kg/day, respectively) for at least 4 weeks. The positive control group (10 males) was administered cyclophosphamide (4.5 mg/kg bw/day) by gavage for the same duration. During the study, clinical condition, bodyweight, food and water consumption, organ weight, and macroscopic pathology were evaluated. Six days before necropsy, all animals in all groups received a single intraperitoneally dose of sheep red blood cells (SRBC) 0.5 mL/animal ( $4 \times 10^8$  SRBC /mL). At sacrifice, selected organs were removed and weighed (spleen, liver, thyroid gland, adrenal gland and thymus). The SRBC-specific immunoglobulin response was measured with Enzyme Linked Immunosorbent Assay (ELISA). Plasma thyroid hormone levels were also measured.

There were no premature deaths, and no treatment-related clinical signs. No treatment related effects on food and water consumption and mean body weights at low (10 ppm) and mid dose (50 ppm) groups. However, decreased food consumption over the entire study period was found in high dose group (250 ppm). Decreased mean body weight and body weight changes ( $p \leq 0.01$ ) were also found in high dose group. Thyroxin ( $T_4$ ) levels were statistically significantly decreased ( $p < 0.01$ ) in all treated groups. Increased relative thyroid gland weight, increased TSH level and moderate to severe follicular hypertrophy/hyperplasia in thyroid glands were found in the high dose group. Mean absolute and relative thymus gland weights were statistically significantly decreased ( $p \leq 0.01$  and  $p \leq 0.05$ , respectively) in mid and high dose groups; although it was reported that no associated histopathological changes were found. Positive control group treated with cyclophosphamide had statistically significant decreases ( $p < 0.01$ ) in absolute and relative spleen and thymus weights.

**The systemic LOAEL was 10 ppm (equivalent to 1 mg/kg/day) based on decreased thyroid hormone levels. The systemic NOAEL was < 10 ppm (equivalent to < 1 mg/kg/day).**

There were no statistically significant differences observed in anti-SRBC IgM levels in treated groups when compare to the vehicle control group. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC antibody response. Positive control group had statistically significant ( $p < 0.05$ ) decrease of the

antibody response. This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for Ethylenethiourea does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system. Under HED guidance a NK cell activity assay is not required at this time.

**The NOAEL for immunotoxicity was 250 ppm (equivalent to 19 mg/kg /day), the highest doses tested. The immunotoxicity LOAEL was not established.**

This immunotoxicity study is classified **acceptable/guideline** and satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the rat.

EPA Primary Reviewer: Khin Swe Oo, MD, DABTSignature: [Signature]

TEB, Health Effects Division (7509P)

Date: 2/13/2013EPA Secondary Reviewer: Yung G. Yang, Ph.D.Signature: [Signature]

Risk Assessment Branch VI, Health Effects Division (7509P)

Date: 2/13/2013

Template version 09/11

TXR #: 0056580

**DATA EVALUATION RECORD****STUDY TYPE:** Immunotoxicity [dietary] - Rat; OPPTS 870.7800**PC CODE:** 600016**DP BARCODE:** D402788**TEST MATERIAL (PURITY):** Ethylenethiourea (100 %, a.i.)**SYNONYMS:** 1,3-Ethylene-2-thiourea; N,N-Ethylenethiourea; ETU.**CITATIONS:**

Strauss V., Groters S., Becker M., et al. (2012). Ethylenethiourea: Immunotoxicity study in male Wistar rats, administration via the diet for 4 weeks. Project No. 42C0533/00S010. Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen, Germany. MRID# 48794502. April 2012. Unpublished.

Strauss V., Groters S., Becker M., et al. (2011). Ethylenethiourea: Test study in Wistar rats, administration via the diet for 2 weeks. Project No. 10C0533/00S09. Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen, Germany. MRID# 48794501. October 19, 2011. Unpublished.

Ollinger J., (2012). Assessment of the Immunotoxic Potential of Ethylenethiourea. Ollinger Consulting LLC, c/o EDBC/ETU Task force. Project no. ETU-WOE-2012. MRID# 48807701. April 6, 2012. Unpublished.

**SPONSOR:** EDBC/ETU Task Force, 600 13<sup>th</sup> Street, N.W., Washington DC 20006, USA.

**EXECUTIVE SUMMARY:** In an immunotoxicity study (MRID #48794502), Ethylenethiourea (100 % w/w a.i., Batch no. 07202TD) was administered to Male Wistar rats (10/dose) in the diet at dose levels of 0, 10, 50, or 250 ppm (equivalent to 0, 1, 4, and 19 mg/kg/day, respectively) for at least 4 weeks. The positive control group (10 males) was administered cyclophosphamide (4.5 mg/kg bw/day) by gavage for the same duration. During the study, clinical condition, bodyweight, food and water consumption, organ weight, and macroscopic pathology were evaluated. Six days before necropsy, all animals in all groups received a single intraperitoneally dose of sheep red blood cells (SRBC) 0.5 mL/animal ( $4 \times 10^8$  SRBC /mL). At sacrifice, selected organs were removed and weighed (spleen, liver, thyroid gland, adrenal gland and thymus). The SRBC-specific immunoglobulin response was measured with Enzyme Linked Immunosorbent Assay (ELISA). Plasma thyroid hormone levels were also measured.

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in high dose group (250 ppm). Decreased mean body weight and body weight changes ( $p \leq 0.01$ ) were also found in high dose group. Thyroxin ( $T_4$ ) levels were statistically significantly decreased ( $p < 0.01$ ) in all treated groups. Increased relative thyroid gland weight, increased TSH level and moderate to severe follicular hypertrophy/hyperplasia in thyroid glands were found in the high dose group. Mean absolute and relative thymus gland weights were statistically significantly decreased ( $p \leq 0.01$  and  $p \leq 0.05$ , respectively) in mid and high dose groups; although it was reported that no associated histopathological changes were found. Positive control group treated with cyclophosphamide had statistically significant decreases ( $p < 0.01$ ) in absolute and relative spleen and thymus weights.

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**The NOAEL for immunotoxicity was 250 ppm (equivalent to 19 mg/kg /day), the highest doses tested. The immunotoxicity LOAEL was not established.**

This immunotoxicity study is classified **acceptable/guideline** and satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the rat

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

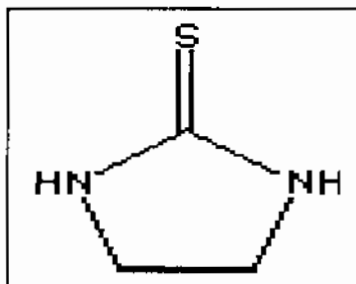
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**I. MATERIALS AND METHODS****A. MATERIALS:****1. Test material:**

Ethylenethiourea

Description: Solid White  
Lot/Batch #: 07202TD  
Purity: 100 %  
Compound Stability: Stable for 10 days at room temperature.  
CAS # of TGAI: 96-45-7

Structure

**2. Vehicle and/or positive control:**

Vehicle: Ground Kliba maintenance diet mouse-rat "GLP", meal from Provimi Kliba SA, Kaiseraugst, Switzerland.

Positive Control: Cyclophosphamide, Batch ID 1362353; 100% purity (from Sigma Aldrich, Taufkirchen, Germany)

**3 Test animals:**

Species:	Rat, Male								
Strain:	CrI:WI(Han)								
Age/weight at treatment initiation :	Approximately 42±1 days old/ weight not reported								
Source:	Charles River, Sulzfeld, Germany.								
Housing:	5 animals/ cage in H-Temp polysulfonate cages with wooden bedding.								
Diet:	Ground Kliba maintenance diet mouse-rat "GLP", meal								
Water:	From water bottles, <i>ad libitum</i>								
Environmental conditions:	<table border="0"><tr><td>Temperature:</td><td>20-24 °C</td></tr><tr><td>Humidity:</td><td>Approximately 30% to 70%</td></tr><tr><td>Air changes:</td><td>15 per hour</td></tr><tr><td>Photoperiod:</td><td>12 hrs dark/ 12 hrs light</td></tr></table>	Temperature:	20-24 °C	Humidity:	Approximately 30% to 70%	Air changes:	15 per hour	Photoperiod:	12 hrs dark/ 12 hrs light
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Humidity:	Approximately 30% to 70%								
Air changes:	15 per hour								
Photoperiod:	12 hrs dark/ 12 hrs light								
Acclimation period:	Not reported.								

**B. STUDY DESIGN:**

1. **In life dates** – Experiment Start: March 20, 2011 End: January 24, 2012.

2. **Animal assignment:** A computerized randomization procedure was used. The bodyweights of animals did not exceed +/- 20% of the overall mean.

Table 1. Study Design

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg/day)	No. of Male animals
1. Vehicle Control	0	0	10
2. Ethylenethiourea	10	1	10
3. Ethylenethiourea	50	4	10
4. Ethylenethiourea	250	19	10
5. Positive Control <sup>b</sup>	0	4.5	10

<sup>a</sup> information was obtained from pages 15, 18 and 23 of the study report<sup>b</sup> Positive Control group received Cyclophosphamide by gavage at 4.5 mg/kg bw/day for 4 weeks

**3. Dose selection:** The dose levels were set based on the results of a two week range-finding study (MRID 48794501) done by same authors in 2011. In that study the thymus weight was statistically significantly ( $p \leq 0.05$ ) decrease at 50 ppm and is correlated with the functional or morphological adverse effect. At 300 ppm, spleen and thymus weight was also statistically significantly decrease ( $p \leq 0.05$ ), and mean body weight change was significantly lower on study day 14 (-26%).

**4. Diet preparation and analysis:** The test substance was measured and mixed with a small amount of food. Depending on test group, the corresponding amounts of food was added to this premix to achieve the desired concentrations. A laboratory mixer was used for mixing.

## Results

### Homogeneity and Concentration control analysis:

Name	Amount [ppm]	Average [ppm]	Nominal Conc [ppm]	Nominal Conc %	Mean %	RSD %
3	9.93		10	99.3		
4	10.25	10.20	10	102.5	102.0	2.0
5	10.42		10	104.2		
6	51.86		50	103.7		
7	52.95	52.08	50	105.9	104.2	1.5
8	51.44		50	102.9		
9	267.70		250	107.1		
10	268.00	269.2	250	107.2	107.7	0.9
11	271.80		250	108.7		

Data obtained from page 179 of the study report

**Stability analysis:** The stability of the test substance was demonstrated over a period of 10 days at room temperature.

**Concentration analysis:** The concentrations of all dose levels were in the ranged from (102.0-107.7 %) of nominal concentrations.

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**5. Statistics:****Test groups 0, 1, 2 and 3**

Parameters	Statistical test	Markers in the tables	References
body weight and body weight change	A comparison of each test group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096-1121  DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482-491

**Test groups 0 and 4**

Parameters	Statistical test	Markers in the tables	References
body weight and body weight change	A comparison of the dose group with the control group was performed using the t-test* (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	WELCH, B.L. (1947): The generalization of Student's problem when several different population variances are involved. Biometrika, 34, 28-35

# the t-test is identical to the Dunnett test in the case of 1 test group only

Clinical pathology parameters were analyzed with KRUSKAL-WALLIS test, if the p value was equal or less than 0.05, WILCOXON test was performed.

**C. METHODS:**

- 1. Observations:** Animals were inspected at least once a day for treatment related health effects. Detailed physical examination was done once every week.
- 2. Body weight:** Body weights were recorded weekly before treatment started, on the day of treatment commenced and twice weekly throughout the study period and before necropsy.
- 3. Food/water consumption and compound intake:** During the test period, food intake and water consumption were recorded weekly.
- 4. Sacrifice and pathology:** On the day of sacrifice, the blood samples were collected under isoflurane anesthesia from the retro orbital sinus after fasting for 16 -20 hours. Animals were

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sacrificed by exsanguination while under deep anesthesia.

a. **Gross necropsy:** A complete necropsy was conducted on all animals. Following gross examination, spleen, liver, thyroid gland, adrenal gland and thymus, weights were recorded and fixed in 4% buffered formaldehyde solution.

b. **Tissue preparation/histopathology:** After fixation and histo - technical processing, above organs were examined without light microscopy in low and mid dose (10 and 50 ppm) test groups.

## 5. Immunotoxicity:

a. **Enzyme-linked Immunosorbent Assay (ELISA):** Six days before necropsy, all animals in all groups received a single intraperitoneal dose of antigen SRBC  $4 \times 10^8$  per mL (0.5 mL/animal). ELISA was performed to determine the level of SRBC- specific immunoglobulin M response to the antigen administration and Sunrise MTP-reader, Tecan AG, Maennedorf, Switzerland was used.

b. **NK cell Assay:** Did not perform NK cell assay.

6. **Hormones:** TSH level was measured with direct, competitive radioimmuno assay. Total triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) levels were measured with ELISA.

## II. RESULTS:

### A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

2. **Mortality:** There were no unscheduled mortalities during the study.

B. **Body weight and weight gain:** Mean body weights in low and mid dose groups were not statistically significantly differences from the vehicle control group (Table 2). However, the mean absolute body weight gain at 50 ppm was significantly lower ( $p < 0.01$ ) on study days 7, 11, 14, 18 and 25 with a maximum of -13% on study day 7 when compare with the vehicle control group (Table 3). Mean body weight and body weight gain were statistically significantly reduced ( $p < 0.01$ ) in 250 ppm group throughout the study period. These findings were considered to be treatment related. The mean body weights and body weight changes in the positive control group were significantly reduced from the vehicle control group with maximum reduction (-8%) on study day 28 ( $p < 0.01$ ).

TABLE 2. Mean body weights (g)

		0/M	1/M	2/M	3/M
day 0	Mean	141.4 n	141.1	141.1	141.4
	S.d.	6.3	6.6	6.5	5.4
	N	10	10	10	10
	Deviation Vs Control		-0.2	-0.2	0.0
day 4	Mean	187.7 n	187.0	184.6	184.4
	S.d.	7.7	8.2	7.5	7.4
	N	10	10	10	10
	Deviation Vs Control		-0.4	-1.8	-2.0
day 7	Mean	184.6 n	183.2	178.8	180.5
	S.d.	8.4	9.5	8.0	6.6
	N	10	10	10	10
	Deviation Vs Control		-0.8	-3.2	-2.2
day 11	Mean	208.5 n	206.2	199.4	198.1 *
	S.d.	8.6	10.8	9.7	10.8
	N	10	10	10	10
	Deviation Vs Control		-1.1	-4.3	-6.9
day 14	Mean	226.3 n	223.3	215.2	208.8 **
	S.d.	10.0	11.7	9.8	12.2
	N	10	10	10	10
	Deviation Vs Control		-1.3	-4.9	-7.8
day 18	Mean	247.8 n	245.9	235.2	224.9 **
	S.d.	10.8	12.0	11.9	14.5
	N	10	10	10	10
	Deviation Vs Control		-0.8	-5.1	-9.2
day 21	Mean	258.9 n	258.1	249.5	235.0 **
	S.d.	12.7	12.1	11.9	15.8
	N	10	10	10	10
	Deviation Vs Control		-0.7	-4.0	-9.6
day 25	Mean	278.4 n	275.5	266.9	246.9 **
	S.d.	13.8	13.8	12.0	16.8
	N	10	10	10	10
	Deviation Vs Control		-1.1	-4.5	-11.3
day 28	Mean	288.0 n	287.3	276.4	255.1 **
	S.d.	14.7	14.6	12.7	17.8
	N	10	10	10	10
	Deviation Vs Control		-0.3	-4.0	-11.4

Statistic Profile = Dunnett test (two-sided), \* p &lt;= 0.05, \*\* p &lt;= 0.01, X = Group excluded from statistics

Data obtained from page 54 in the study report.

0 = vehicle control; 1 = 10ppm; 2 = 50 ppm; 3 = 250 ppm

Table 3. Mean Absolute Body Weight Gain (g)

		0/M	1/M	2/M	3/M
d 0 -> 4	Mean	26.3 n	25.9	23.5	23.0 *
	S.d.	2.1	2.5	3.1	3.6
	N	10	10	10	10
	Deviation Vs Control		-1.6	-10.8	-12.4
d 0 -> 7	Mean	43.2 n	42.0	37.5 **	38.1
	S.d.	3.0	3.8	3.2	5.0
	N	10	10	10	10
	Deviation Vs Control		-2.7	-13.2	-9.5
d 0 -> 11	Mean	87.1 n	86.0	58.3 **	54.8 **
	S.d.	4.8	5.7	5.0	7.8
	N	10	10	10	10
	Deviation Vs Control		-3.0	-13.1	-18.4
d 0 -> 14	Mean	85.0 n	82.2	74.0 **	67.4 **
	S.d.	5.3	6.5	4.9	8.1
	N	10	10	10	10
	Deviation Vs Control		-3.2	-12.8	-20.8
d 0 -> 18	Mean	106.4 n	104.8	94.0 **	83.5 **
	S.d.	6.7	8.9	6.7	12.1
	N	10	10	10	10
	Deviation Vs Control		-1.5	-11.8	-21.5
d 0 -> 21	Mean	118.5 n	117.0	108.4	93.7 **
	S.d.	8.7	7.2	7.0	13.4
	N	10	10	10	10
	Deviation Vs Control		-1.3	-8.6	-21.0
d 0 -> 25	Mean	137.0 n	134.3	124.7 *	105.5 **
	S.d.	10.6	8.9	7.6	14.4
	N	10	10	10	10
	Deviation Vs Control		-2.0	-8.0	-23.0
d 0 -> 28	Mean	148.8 n	146.2	135.3	113.7 **
	S.d.	11.5	9.9	7.8	15.8
	N	10	10	10	10
	Deviation Vs Control		-0.3	-7.7	-22.5

Statistic Profile = Dunnett test (two-sided), \* p &lt;= 0.05, \*\* p &lt;= 0.01, X = Group excluded from statistics

Data obtained from page 56 of the study report

0 = vehicle control; 1 = 10ppm; 2 = 50 ppm; 3 = 250 ppm

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**C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:**

1. **Food consumption/ Food Efficiency:** Mean food consumption was statistically significant decreased throughout the study with maximum of 35% on study day 28, in the 250 ppm (highest dose) group when compared to the vehicle control group (Table 4).

Table 4. Mean Food Consumption/day (g)		0/M	1/M	2/M	3/M
d 6 -> 7	Mean (g)	17.3	16.6	15.6	15.1
	S.d.	1.3	0.8	2.0	1.5
	N	2	2	2	2
	Deviation Vs Control		-4.0	-9.8	-12.4
d 13 -> 14	Mean (g)	20.9	20.1	19.3	18.6
	S.d.	0.1	2.5	1.8	1.0
	N	2	2	2	2
	Deviation Vs Control		-3.8	-7.9	-20.8
d 20 -> 21	Mean (g)	21.2	20.5	19.5	16.9
	S.d.	1.1	0.0	1.2	0.4
	N	2	2	2	2
	Deviation Vs Control		-3.5	-8.5	-20.7
d 27 -> 28	Mean (g)	25.5	23.0	24.4	18.5
	S.d.	3.5	0.3	1.8	1.3
	N	2	2	2	2
	Deviation Vs Control		-9.8	-4.5	-35.3

Data obtained from page 52 of the study report

2. **Compound consumption:** The compound consumption in each group was shown in Table 1.

- D. **GROSS NECROPSY:** During the gross observations no treatment related visible lesions were found at any concentration.

1. **Organ weight:** The mean absolute and relative thymus weights were statistically significantly decreased in group 2 and 3 (50 and 250 ppm groups); however it was reported that there were no associated histopathological changes. Mean relative liver weights were statistically significantly increased ( $p < 0.05$ ) in mid dose and high dose groups. The mean relative thyroid gland weight was statistically significantly higher ( $p < 0.01$ ) in the high dose group (Table 6). Positive control group treated with cyclophosphamide had statistically significant decreased ( $p < 0.01$ ) in absolute and relative spleen and thymus weights.

Table 5. Absolute Organ Weights, Mean Values			F1				
Sacrifice	Sex	Group	M	0	1	2	3
Terminal body weight	g	M	264.88	264.02	254.03	235.54	**
	% dev		100	100	96	89	
	SD		14.255	12.05	12.762	16.542	
	n		10	10	10	10	
Adrenal glands	mg	M	54.6	58.3	59.3	48.3	
	% dev		100	107	109	88	
	SD		7.351	12.41	7.273	6.273	
	n		10	10	10	10	
Liver	g	M	7.173	7.32	7.292	6.82	
	% dev		100	102	102	95	
	SD		0.402	0.36	0.464	0.755	
	n		10	10	10	10	
Spleen	g	M	0.525	0.58	0.583	0.432	**
	% dev		100	110	111	82	
	SD		0.067	0.076	0.077	0.065	
	n		10	10	10	10	
Thymus	mg	M	525.2	514.4	415.1	403.5	**
	% dev		100	98	79	77	
	SD		86.152	67.426	65.261	58.291	
	n		10	10	10	10	
Thyroid glands	mg	M	18.7	20.0	19.4	41.5	**
	% dev		100	107	104	222	
	SD		2.627	2.789	2.413	9.071	
	n		10	10	10	10	

\*:  $P < 0.05$ , \*\*:  $P < 0.01$   
Kruskal-Wallis H and Wilcoxon test, two sided

Data obtained from page 63 of the study report

Table 6: Relative Organ Weights, Mean Values

Sacrifice Sex Group			F1 M 0	1	2	3
Terminal body weight	%	M	100.0	100.0	100.0	100.0
	% dev		100	100	100	100
	n		10	10	10	10
Adrenal glands	%	M	0.021	0.022	0.023	0.021
	% dev		100	107	113	99
	SD		0.003	0.004	0.003	0.002
	n		10	10	10	10
Liver	%	M	2.71	2.774	2.871*	2.892*
	% dev		100	102	106	107
	SD		0.105	0.108	0.139	0.205
	n		10	10	10	10
Spleen	%	M	0.198	0.22	0.229*	0.184
	% dev		100	111	116	93
	SD		0.019	0.028	0.027	0.028
	n		10	10	10	10
Thymus	%	M	0.199	0.195	0.164*	0.172*
	% dev		100	98	82	86
	SD		0.036	0.023	0.027	0.024
	n		10	10	10	10
Thyroid glands	%	M	0.007	0.008	0.008	0.018**
	% dev		100	107	108	248
	SD		0.001	0.001	0.001	0.003
	n		10	10	10	10

\*: P &lt;= 0.05, \*\*: P &lt;= 0.01

Kruskal-Wallis H and Wilcoxon test, two sided

Data obtained from page 65 of the study report.

**2. Histopathology:** The high dose group (250 ppm) had moderate to severe follicular hypertrophy/hyperplasia in thyroid glands. It was reported that the changes were treatment related.

## E. IMMUNOTOXICITY TESTS:

**a. Enzyme - linked Immunosorbent Assay (ELISA):** Anti-SRBC IgM levels in treated groups when compare to the vehicle control group were not statistically significant. However, it should be noted that the Standard Deviations were very high, which may lower the quality of test performed and the resulting data. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC antibody response. Positive control group had statistically significant ( $p < 0.01$ ) decrease of the antibody response (Table 7, Figures 1). This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

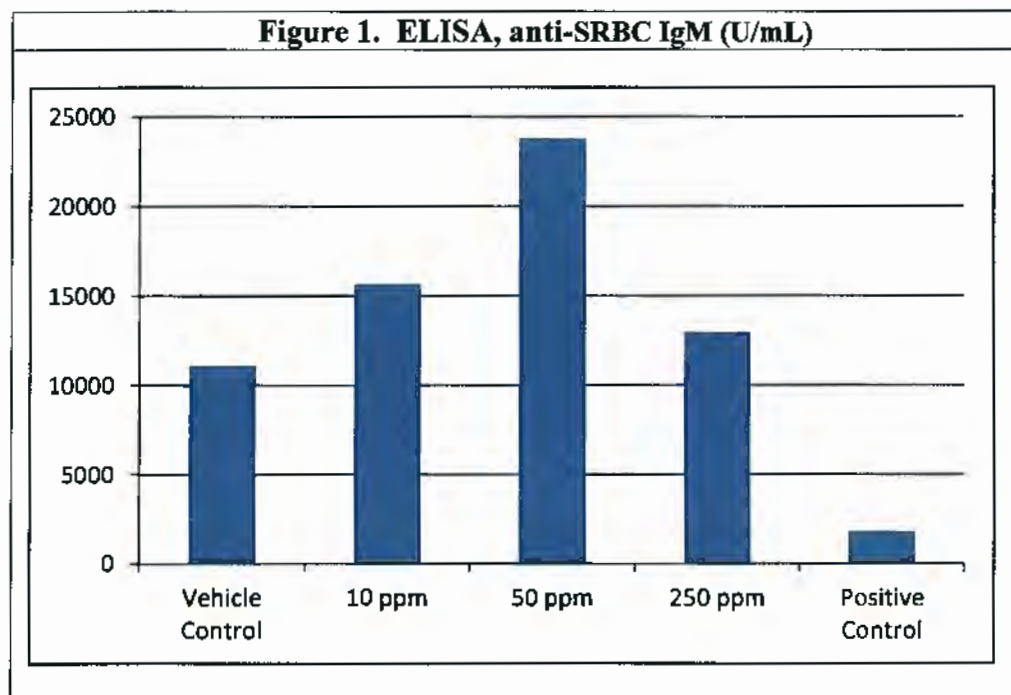
Table 7. Anti-SRBC IgM (U/mL), Mean±SD

Vehicle control	10 ppm	50 ppm	250 ppm	Positive Control Group (Cyclophosphamide)
11,073±11,775	15624±7866	23761±19808	12938±6208	1774±860**

Data obtained from pages 59 and 60 of study report

\*\*  $p < 0.01$ 

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Data obtained from pages 59 and 60 of the study report

Group 1 = Vehicle Control; Group 2 = 10 ppm; Group 3 = 50 ppm; Group 4 = 250 ppm.  
Group 5 = Positive control (Cyclophosphamide treated group).

**b. NK cell assay:** Did not perform NK cell assay.

**F. Thyroid Hormone levels:** T<sub>4</sub> levels were statistically significantly decreased ( $p < 0.01$ ) with dose-response relationship in all dose groups. The positive control group had no changes in T<sub>4</sub> levels. TSH levels were only increased in the high dose group (250 ppm). T<sub>3</sub> levels were not altered in any dose group (Table 8).

**Table 8. Thyroid hormone levels**

		0/M	1/M	2/M	3/M
T3 [nmol/L] day 29	Mean	1.54 k	1.34	1.30	1.83
	S.d.	0.25	0.19	0.17	0.34
	N	10	10	10	10
	Median	1.45	1.38	1.33	1.81
T4 [nmol/L] day 29	Mean	84.72 v	53.42 **	41.33 **	28.70 **
	S.d.	8.90	5.23	4.73	8.77
	N	10	10	10	10
	Median	66.16	52.24	42.84	24.96
TSH [µg/L] day 29	Mean	2.61 v	3.89	4.30	91.07 **
	S.d.	1.38	1.93	2.25	60.43
	N	10	10	10	10
	Median	3.06	3.93	3.79	69.84

Statistic Profile = Kruskal-Wallis + Wilcoxon test (two-sided), \*  $p < 0.05$ , \*\*  $p < 0.01$ , X = Group excluded from statistics  
k=KRUSKAL-WALLIS; v=KRUSKAL-WALLIS-WILCOX

Data obtained from page 61 of the study report.

### III. DISCUSSION AND CONCLUSIONS:

**A. INVESTIGATORS' CONCLUSIONS:** It was reported that the Ethylenethiourea did not reveal any signs of immunotoxicity when administered in the diet over a period of 4 weeks to male Wister rats. The NOAEL for immunotoxicity was 250 ppm (19 mg/kg/day). The systemic NOAEL was below 10 ppm due to decreased thyroid hormone levels.

**B. REVIEWER COMMENTS:** There were no premature deaths, and no treatment-related clinical signs. No treatment related effects on food and water consumption and mean body weights at low (10 ppm) and mid dose (50 ppm) groups. However, decreased food consumption over the entire study period was found in high dose group (250 ppm). Decreased mean body weight and body weight changes ( $p \leq 0.01$ ) were also found in high dose group. Thyroxin ( $T_4$ ) levels were statistically significantly decreased ( $p < 0.01$ ) in all treated groups. Increased relative thyroid gland weight, increased TSH level and moderate to severe follicular hypertrophy/hyperplasia in thyroid glands were found in the high dose group. Mean absolute and relative thymus gland weights were statistically significantly decreased ( $p \leq 0.01$  and  $p \leq 0.05$ , respectively) in mid and high dose groups; although it was reported that no associated histopathological changes were found. Positive control group treated with cyclophosphamide had statistically significant decreased ( $p < 0.01$ ) in absolute and relative spleen and thymus weights.

**The systemic LOAEL was 10 ppm (equivalent to 1 mg/kg/day) based on decreased thyroid hormone levels. The systemic NOAEL was < 10 ppm (equivalent to < 1 mg/kg/day).**

There were no statistically significant differences observed in anti-SRBC IgM levels in treated groups when compare to the vehicle control group. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC antibody response. Positive control group had statistically significant ( $p < 0.05$ ) decrease of the antibody response (Table 7, Figures 1). This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for Ethylenethiourea does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system. Under HED guidance a NK cell activity assay is not required at this time.

**The NOAEL for immunotoxicity was 250 ppm (equivalent to 19 mg/kg /day), the highest doses tested. The immunotoxicity LOAEL was not established.**

**C. STUDY DEFICIENCIES:** No major deficiencies were noted.